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=> d his

     (FILE 'HOME' ENTERED AT 13:05:15 ON 02 NOV 2006)

     FILE 'USPATFULL' ENTERED AT 13:05:21 ON 02 NOV 2006

     FILE 'USPATFULL, CAPLUS' ENTERED AT 13:05:28 ON 02 NOV 2006
L1         30594 FILE USPATFULL
L2         34388 FILE CAPLUS
     TOTAL FOR ALL FILES
L3         64982 S EXTRACT? (10A) CRUDE?
L4            93 FILE USPATFULL
L5          1590 FILE CAPLUS
     TOTAL FOR ALL FILES
L6          1683 S (ASIMINA OR ANNONA OR GONIOTHALAMUS OR UVARIA OR DISEPALUM OR
L7        179016 FILE USPATFULL
L8        325687 FILE CAPLUS
     TOTAL FOR ALL FILES
L9        504703 S TWIG OR FRUIT OR SEED OR BARK
L10       689459 FILE USPATFULL
L11      1160498 FILE CAPLUS
     TOTAL FOR ALL FILES
L12     1849957 S SIEV? OR PULVERIZ? OR PERCOLAT? OR ALCOHOL? OR ETHANOL?
L13          19 FILE USPATFULL
L14           2 FILE CAPLUS
     TOTAL FOR ALL FILES
L15          21 S L3 AND L6 AND L9 AND L12
L16           2 FILE USPATFULL
L17           0 FILE CAPLUS
     TOTAL FOR ALL FILES
L18           2 S L15 AND (SPRAY (3A) (DRY? OR DRIED?))

=> s l15 and ((dry? or dried?))
L19          18 FILE USPATFULL
L20           0 FILE CAPLUS

TOTAL FOR ALL FILES
L21          18 L15 AND ((DRY? OR DRIED?))

=> d 10-18 kwic, ibib
```

TD [0029] A participant suffering from stage four breast cancer started taking **crude extract** capsules, without changing any other treatment protocol. After just six weeks of taking the capsules, a 50% percent reduction in. . .

DETD . . . chemotherapy without success. During this time, the participant was limited to a wheelchair or bedridden. Within two months of taking **crude extract** capsules his tumor markers improved, showing a decrease from 275 to 222. The participant had a weight gain of five pounds and did not suffer from side effects of the **crude extract** capsules. The participant is now able to walk on his own.

DETD [0031] A participant suffering from stage four melanoma started taking **crude extract** capsules in November 2002. The melanoma had previously metastasized to the lungs causing great difficulty while breathing. The participant experienced easier breathing within days of taking **crude extract** capsules. The participant has since been able to get out of bed and even progressed to riding a bike, walking. . .

DETD . . . 0-3.5. A 56 year old participant suffering from prostate cancer, that was confirmed by biopsy, started taking four 12.5 mg **crude extract** capsules per day in October 2002. His PSA levels dropped from 3.85 on October 2002 to 2.08 on December 2002. This participant continued to take **crude extract** capsules until April 2003.

DETD [0033] A participant suffering from stage four metastasizing prostate cancer started taking **crude extract** capsules. There was a distinct reduction in the tumor masses within six weeks of taking the capsules, although he was. . .

DETD [0034] The examples listed above particularly show the efficacy of the **crude extract**. Table 1 is a complete list of the experiment results.

TABLE 1

Progress of patients with clinical cancer taking capsules containing **crude extract**.

| Number | Cancer Type | Comments |
|---|---|---|
| 1 | Bone cancer | Started at the end of January. December 2002 Alk-Phos test was 242. Feb. 22, 2003. . . |

CLM What is claimed is:

1. A composition comprising a **crude extract** containing at least one annonaceous acetogenin, wherein the **crude extract** is prepared from at least one species in the group consisting of the annonaceous genera **Asimina**, **Annona**, **Goniothalamus**, **Uvaria**, **Disepalum**, **Xylopia**, and **Rollinia**.

2. A composition in accordance with claim 1, wherein the **crude extract** is in a capsule form.

3. A composition in accordance with claim 1, wherein the **crude extract** is in tablet form.

4. A composition in accordance with claim 1, wherein the **crude extract** is in tincture or liquid form.

5. A composition in accordance with claim 1, wherein said species is **Asimina** triloba.

6. A composition in accordance with claim 5, wherein the **crude extract** is prepared from **twigs** of the **Asimina** triloba.

7. A method for **extracting** a **crude extract**, comprising the steps of: (a) obtaining one or more **twig**, unripe **fruit**, **seed**, **bark** or other bioactive plant part, or any combination thereof, the one or more **twig**, unripe **fruit**, **seed**, **bark** or other bioactive plant part being of a genus selected from the group consisting of **Asimina**, **Annona**, **Goniothalamus**, **Uvaria**, **Disepalum**, **Xylopia**, and **Rollinia**; (b) drying the one or more **twig**, unripe **fruit**, **seed**, **bark** or other bioactive plant part in a forced air drier at less than 50° C. to form a mass; (c) placing the mass in a **sieve** to form a **sieved** product; (d) **pulverizing** the **sieved** product in a chipper to form a **pulverized** product; (e) placing the **pulverized** product in a **percolator**; (f) performing at least one water extraction on the **pulverized** product; (g) performing at least one **ethanol** extraction on the **pulverized** product to provide an **ethanolic** extract; (h) concentrating the **ethanolic** extract, in vacuo, at about 50° C., to form a syrup; (i) allowing the syrup to settle into a **crude extract** layer and a water layer; (j) removing the water layer from the **crude extract** layer to form a concentrate; and (k) **spray drying** the concentrate onto an inert carrier to facilitate encapsulation or tableting.

8. The method of claim 7, further comprising the step of standardizing the **crude extract** for zero percent moisture and an LC.sub.50 value of 0.5 ppm in a BST.

9. The method of claim 7, further comprising the step of standardizing the **crude extract** for a range of 10-40% moisture, and an LC.sub.50 value in a range of 0.2-0.8 ppm in a BST.

13. A method for determining a patient's tolerance to a **crude extract** including the steps of: (a) ingesting 12.5 mg of a composition comprising a **crude extract** containing at least one annonaceous acetogenin, wherein said **crude extract** is prepared from at least one species from the group consisting of the annonaceous genera **Asimina**, **Annona**, **Goniothalamus**, **Uvaria**, **Disepalum**, **Xylopia**, or **Rollinia**., on day one; (b) ingesting 25 mg of the **crude extract** composition on day two; (c) ingesting 37.5 mg of the **crude extract** composition on day three; and (d) ingesting 50 mg of the **crude extract** on day four.

14. The method of claim 13, further comprising the steps of: (a) evaluating the patient's tolerance daily after ingesting the **crude extract**.


| | | | | |
|---|---|---|---|---|
| ACCESSION NUMBER: | 2004:133048 USPATFULL | | | |
| TITLE: | Control of cancer with annonaceous extracts | | | |
| INVENTOR(S): | McLaughlin, Jerry Loren, West Lafayette, IN, UNITED STATES<br>Benson, Gina Bellcssa, Provo, UT, UNITED STATES | | | |
| | NUMBER | KIND | DATE | |
| PATENT INFORMATION: | US 2004101584 | A1 | 20040527 | |
| APPLICATION INFO.: | US 2003-717746 | A1 | 20031120 | (10) |
| | NUMBER | | DATE | |

| | |
|---|---|
| PRIORITY INFORMATION: | US 2002-428602P 20021122 (60) |
| DOCUMENT TYPE: | Utility |
| FILE SEGMENT: | APPLICATION |
| LEGAL REPRESENTATIVE: | Vanessa B. Pierce, Parsons Behle & Latimer, Suite 1800, 201 South Main Street, Salt Lake City, UT, 84111-2218 |
| NUMBER OF CLAIMS: | 14 |
| EXEMPLARY CLAIM: | 1 |
| NUMBER OF DRAWINGS: | 1 Drawing Page(s) |
| LINE COUNT: | 683 |

the extraction, a small amount of the material is prepared for investigation purposes. Ten (10) grams of the powdered **bark** of each species is ground (0.2 mm **sieve**), combined with five (5) cubic centimetres of limejuice and defatted with hexane (200 ml) overnight at room temperature. The plant. . . The filtrates are dried under vacuum and the residue stored at room temperature until testing. Tannins are removed from the **crude** methanolic **extracts** using Sephadex LH-20 exclusion chromatography. Methylene chloride and methanol extract are then performed according to standard methods.

SUMM In yet another embodiment, equal proportions of dried Z. gilletti and A. leiocarpus stem **barks** (1.0 g) are mixed with 250 ml of 30% methanol in water in a beaker. The mixture is boiled for. . .

CLM What is claimed is:

. . . The method of claim 24 wherein said liquid organic extractant is selected from the group comprising: water, acetone, toluene, benzene, **ethanol**, heptane, hexane, pentanone, methanol, propanol, isopropanol, ethyl acetate, diethyl ether, trichloroethane, methyl ethyl ketone, n-butanol, 1,2-dichloroethane, dichloromethane, chloroform and mixtures. . .

. . . is achieved by reducing the temperature of said extractant to solidify said biomass extract; recovering said solidified biomass extract by **spray-drying**, freeze-**drying** or concentrating-drying to obtain dried powder biomass extract.

. . . to 6 hours; reducing the temperature of said mixture to solidify said biomass extract; recovering said solidified biomass extract by **spray-drying**, freeze-**drying** or concentrating-drying to obtain dried powder biomass extract.


| | | | | |
|---|---|---|---|---|
| ACCESSION NUMBER: | 2005:305468 USPATFULL | | | |
| TITLE: | Compositions comprising natural agents for the treatment of HIV-associated opportunistic infections and complications and methods for preparing and using compositions comprising natural agents | | | |
| INVENTOR(S): | Ashiagbor, Kwame Titus, Accra, GHANA<br>Ashiagbor, Stephen, Accra, GHANA<br>Wutoh, Anthony K., Upper Marlboro, MD, UNITED STATES<br>Kallia, Yaw Foster, Accra, GHANA<br>Wutoh, Rita Delores, Upper Marlboro, MD, UNITED STATES<br>Wutoh, Jeffrey K., Brookville, MD, UNITED STATES<br>Aidoo, Elnora, Accra, GHANA | | | |
| | NUMBER | KIND | DATE | |
| PATENT INFORMATION: | US 2005266105 | A1 | 20051201 | |
| APPLICATION INFO.: | US 2005-62769 | A1 | 20050222 | (11) |
| | NUMBER | | DATE | |
| PRIORITY INFORMATION: | US 2004-545508P | | 20040219 | (60) |
| DOCUMENT TYPE: | Utility | | | |
| FILE SEGMENT: | APPLICATION | | | |
| LEGAL REPRESENTATIVE: | ANTHONY K. WUTOH, 17340 Queen Ann Road, Upper Marlboro, MD, 20774, US | | | |
| NUMBER OF CLAIMS: | 60 | | | |
| EXEMPLARY CLAIM: | 1 | | | |
| NUMBER OF DRAWINGS: | 1 Drawing Page(s) | | | |
| LINE COUNT: | 1418 | | | |

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

SUMM . . . al., U.S. Pat. No. 4,721,727, disclosed a new member of this series referred to as asimicin. Asimicin was isolated from **Asimina** triloba Dunal. (Annonaceae) and is characterized by two hydroxyl groups in the R.sup.2 and R.sup.3 positions, two hydrogens in the. . . et al., U.S. Pat. No. 4,721,727]. An uncharacterized pesticidal substance called annonin and a process for its isolation from the **seeds** of **Annona** souamosa (Annonaceae) has been patented by Moeschler et al. [U.S. Pat. No. 4,689,232].

SUMM Pettit et al. (Can. J. Chem., 65: 1433-1435 (1987)] isolated a diastereomer of asimicin from **Rollinia** mucosa (Annonaceae). This diastereomer is called **rolliniastatin**. Its stereochemistry, which was revealed by the first X-ray crystallographic analysis of this type of compound, is threo, cis, threo,. . . as analyzed by the .sup.1 H nmr analysis method developed by Hoye et al. [J. Am. Chem. Soc., 109: 4402-4403(1987)]. **Rollinia** mucosa has been known in primitive medical practices of Indonesia and the West Indies as a treatment for tumors. Biological evaluation of **rolliniastatin** showed PS activities: 28% life extension and ED.sub.50 4.5+10.sup.-5 μg/m in cell culture.

SUMM During the screening of plants in our laboratory, we have unexpectedly discovered that **Annona** bullata Rich. in the Annonaceae family has noteworthy activities in the BST (brine shrimp lethality test), PD (crown gall antitumor. . .

SUMM The starting material for use in the invention is the **bark** of **Annona** bullata Rich. (Annonaceae), and it is considered likely, by the screening of other parts of the plant, that other tissues such as **twigs**, wood, roots, **seeds** and leaves would also contain extractable quantities of the subject compounds.

SUMM The **bark** material is prepared for extraction by grinding in a conventional mill to a suitable particle size, usually in the range. . . about 0.001-3 mm. in diameter, and more preferably in the range of 0.1-2 mm. The ground material is extracted by **percolating** with 95% EtOH. The **ethanol** solubles are concentrated to remove the bulk of the solvent, at least to the point of reducing the extract to. . . partitioned between water and a water-immiscible solvent, such as chloroform, in order to remove the water solubles which are freeze **dried** and labelled F002. The chloroform solubles are recovered as a syrup residue using a solvent evaporator and labelled as F003. The insoluble interface was **dried** at ambient temperature and labelled F004. F003 then is partitioned between hexane and 90% aqueous MeOH in order to remove hexane solubles which are vacuum **dried** and labelled as F006. The 90% aqueous MeOH solubles are recovered by vacuum evaporation to a thick syrup as a **crude** acetogenin-containing **extract** F005.

SUMM Separation and purification of pure acetogenins from the **crude extract** (F005) can be affected by the use of the proper combination of conventional techniques including, for example, column chromatography (CC),. . . desiring to be limited thereto, the details of the separation procedure are illustrated by the following examples. Fractionation of the **ethanolic** extract was guided by assay with the brine shrimp lethality test (BST) and confirmed by assays on tumor cell cultures.. . .

DETD . . . 10, 1, etc. p.p.m. of material in the final brine preparation, assuming complete miscibility in the brine. The vials were **dried** in vacuo, and artificial sea water, prepared from a commercial salt mixture, was added. Ten brine shrimp larva (nauplii), 48-72. . .

DETD Approximately 3.9 kg of **Annona** bullata Rich. (Annonaceae) **bark** (M-06983, PL-103519) was collected by Edward Garvey at the USDA Subtropical Horticulture Research Station, ARS, 13601 Old Culture Rd., Miami, Fla. 33158. The tree originated from **seeds** collected in Cuba in 1933 by Robert M. Grey of Harvard University. Air-**dried bark** was **pulverized** through a 2 mm screen in a Wiley mill. The **pulverized bark** was

extracted by exhaustive **percolation** with 777 liters of 95% EtOH. Vacuum evaporation left 380 g of syrupy residue (F001). F001 was partitioned between CHCl.sub.3 H.sub.2 O (1:1), and the water solubles were freeze **dried** and labelled F002 (11 g). The chloroform solubles were vacuum evaporated to form F003 (181 g). The insoluble interface was air **dried** and labelled F004 (188 g). Then F003 was partitioned between hexane/90% aqueous MeOH (1:1). The 90% MeOH fraction was vacuum. . .

DETD TABLE 1

Bioactivities of Initial Fractions from **Annona** bullata Rich.

| BST | LC.sub.50 mcg/ml | | | | 95% Confidence Interval |
|---|---|---|---|---|---|
| | Protein kinase C | | | | |
| PD | 9KB | 9PS | 9ASK | % Displacement | |
| % Inhibition | | | | | |
| | ED.sub.50 mcg/ml | | | | |
| | | ED.sub.50 mcg/ml reversal | | | |
| | | | 100. . . | | |

DETD TABLE 3

.sup.13 C NMR (CDCl.sub.3) Assignments and Comparisons..sup.a

| Carbon No. | Bullatacin (50 MHz)(1) | Bullatacinone (50 MNz)(2) | **Rolliniastatin** | Asimicin |
|---|---|---|---|---|
| 1 | 174.51s | 178.73s | 174.5s | 174.6s |
| 2 | 131.11s | 44.18d | 131.1s | 131.1s |
| 3.sup.a | 33.23t | 34.41t | 33.2t | 33.4t |
| 4 | 69.91d | 78.86d | 69.9d | 69.9d |
| 5.sup.a | 37.34t | 36.67t | 37.4t. . . | |

DETD . . . of asimicin [Rupprecht et al., Heterocycles, 24: 1197-1201 (1986)], uvaricin [Jolad et al., J. Org. Chem., 47: 3151-3153 (1982)], and **rolliniastatin** [Pettit et al., Can. J. Chem., 65: 1433-1435 (1987)], indicating the common presence of a bistetrahydrofuran moiety as illustrated in. . .

DETD . . . carbon skeleton of bullatacin (1) is the same as that of asimicin [Rupprecht et al., Heterocycles, 24: 1197-1201, (1986)], and **rolliniastatin** [Pettit et al. Can. J. Chem., 65: 1433-1435, (1987) . However, the mp, co-TLC, .sup.1 H nmr, and most importantly,. . .

DETD . . . threo, as illustrated for 1. Similarly, we have determined that asimicin is threo, trans, threo, trans, threo. From X-ray data, **rolliniastatin** is reported to be threo, cis, threo, cis, and erythro [Pettit et al., Can. J. Chem., 65: 1433-1435, (1987)].

DETD . . . chiral centers, carbon 4 and carbon 36, was determined by comparing nmr spectral data with those in the literature for **rolliniastatin** [Pettit et al., Can. J. Chem., 65: 1433-1435, (1987)]. Furthermore, essentially identical CD curves for **rolliniastatin**, asimicin and bullatacin suggested their stereochemical identity in this region. The CD data are given below.

DETD **Rolliniastatin** (c, 0.025; abs. EtOH); [θ].sub.265, 0.00°; [θ].sub.260, -199.04°; [θ].sub.250, -1393.28°; [θ].sub.240, -2587.52°; [θ].sub.235, -2786.56°; [θ].sub.230, -2089.92°; [θ].sub.225, 0.00°; and [θ].sub.220,. . .

DETD Anticancer activity is a potential use even for the **crude**

**extract**. The bioassay results for the lethality of brine shrimp (BST), the inhibition of crown gall tumors on potato discs (PD),. . .

DETD . . . The lack of pesticidal activity for bullatacinone indicates that it did not contribute to the pesticidal activities of the initial **ethanol** extract.

| | |
|---|---|
| ACCESSION NUMBER: | 93:59189 USPATFULL |
| TITLE: | Chemotherapeutically active acetogenins |
| INVENTOR(S): | McLaughlin, Jerry L., W. Lafayette, IN, United States<br>Hui, Yu-Hua, W. Lafayette, IN, United States |
| PATENT ASSIGNEE(S): | Purdue Research Foundation, West Lafayette, IN, United States (U.S. corporation) |

| | NUMBER | KIND | DATE |
|---|---|---|---|
| PATENT INFORMATION: | US 5229419 | | 19930720 |
| APPLICATION INFO.: | US 1992-953759 | | 19920929 (7) |
| RELATED APPLN. INFO.: | Continuation of Ser. No. US 1989-336233, filed on 11 Apr 1989, now abandoned | | |
| DOCUMENT TYPE: | Utility | | |
| FILE SEGMENT: | Granted | | |
| PRIMARY EXAMINER: | Raymond, Richard L. | | |
| ASSISTANT EXAMINER: | Russell, Mark W. | | |
| LEGAL REPRESENTATIVE: | Barnes & Thornburg | | |
| NUMBER OF CLAIMS: | 9 | | |
| EXEMPLARY CLAIM: | 1 | | |
| LINE COUNT: | 880 | | |

CAS INDEXING IS AVAILABLE

L21 ANSWER 17 OF 18 USPATFULL on STN

AB Novel acetogenins isolated from **Asimina** triloba and **Goniothalamus** giganteus of the family Annonaceae and derivatives of those and other acetogenins are described. Bioactive cyclic formaldehyde acetal derivatives are. . . acetogenins having 1,2-, 1,4- or 1,5-diols. A non-adjacent bis-tetrahydrofuran(THF) acetogenin is prepared from an unsaturated mono-THF acetogenin earlier isolated from **Goniothalamus** giganteus. The substantially pure acetogenins and acetogenin derivatives of the invention exhibit cytotoxicity to human solid tumor cell lines equipotent. . .

SUMM . . . in the phyto-chemistry of the Annonaceae has been sparked by the bioactivity-directed isolation of the antileukemic Annonaceous acetogenin, uvaricin, from **Uvaria** acuminata. Acetogenins are C.sub.35 -C.sub.39 compounds and typically contain two long hydrocarbon chains, one of which connects a terminal 2,4-disubstituted-γ-lactone. . .

SUMM . . . present invention there are provided novel, cytotoxic acetogenins and acetogenin derivatives. One group of acetogenins of this invention isolated from **Asimina** triloba are represented by the general formula

SUMM . . .. thereof. The compound wherein --R.sub.3 -- is the divalent group (VI) is denominated goniocin, a naturally occurring acetogenin isolated from **Goniothalamus** giganteus. The compound wherein --R.sub.3 -- is the divalent group (VII) is prepared by epoxidation and subsequent acid-catalyzed cyclization of a previously reported acetogenin, gigantetronenin (also isolated from **Goniothalamus** giganteus), having the above formula wherein --R.sub.3 -- is a divalent group of the formula ##STR5##

DETD Bullatacin is one of the most potent antitumor and pesticidal Annonaceous acetogenins and was first reported and isolated from **Annona** bullata in 1989. The correct absolute configurations of the stereogenic carbinol centers of bullatacin were recently established by .sup.1 H-. . . systems to reduce the ATP levels. Eleven Annonaceous acetogenins have been previously reported from the ETOH extract of the stem **bark** of **Asimina** triloba.
Directed by the brine shrimp lethality test (BST), two related igsomeric acetogenins, the bullatacin threo-trans-threo-trans-erythro from C-15 to C-24. . .

DETD The **bark** of **Asimina** triloba (L.) Dunal was collected from stands growing wild at the Purdue Horticultural Research Farm, West Lafayette, Ind., U.S.A. The. . . was confirmed by Dr. George R. Parker, Department of Forestry and Natural Resources, Purdue University. A voucher specimen of the **bark** is preserved in the pharmacognosy herbarium.

DETD The air-**dried pulverized** stem **bark** (15 kg) was extracted exhaustively (12 days) at room temperature with 95% EtOH (451+4) and vacuum evaporated to yield extract. . .

DETD Asimicin was the first acetogenin isolated from the **seeds** and stem **bark** of the North American paw paw tree, **Asimina** triloba Dunal (Annonaceae). Asimicin has been reported as exhibiting highly potent antitumor and pesticidal activities. Further studies of A. triloba stem **bark** has led to the discovery of additional novel bioactive acetogenins, including the very active adjacent bis-tetrahydrofuran (THF) compound, trilobacin. The. . .

DETD Further activity-directed fractionation of the **ethanolic** extract of the stem **bark**, using the brine shrimp lethality test (BST) to monitor fractionation, has revealed three novel adjacent bis-THF acetogenins, asimin (3), asiminacin. . .

DETD In searching for new bioactive acetogenins from the F005 fraction, which was partitioned from the EtOH extract of the stem **bark**, the more polar column fractions from the most active pools (P7-P9) were investigated. The fraction sample was subjected to open. . .

DETD . . . EtOH and heating. Chromatotron plates (1 or 2 mm) were prepared

with silica gel 60 PF 254 containing gypsum and **dried** at 700 overnight. HPLC was carried out with a Rainin HPLC instrument using the Dynamax software system and a silica. . .

DETD . . . the presence of pyridine. Approximately 10-50 μg of pure compound was placed in a 100 μl conical reaction vial and **dried** in a vacuum desiccator over P.sub.2 O.sub.5 for 24 hrs. The sample was treated with 2 μl pyridine and 20. . .

DETD . . . X.-P.; Miesbauer, L. R., Smith, D. L.; and McLaughlin, J. L., 30, 31, and 32-Hydroxybullatacinones: Bioactive Terminally-Hydroxylated Annonaceous Acetogenins from **Annona** bullata., J. Nat. Prod., 1993, 56, 870-876; MCF-7 (human breast carcinoma) McLaughlin, J. L., Chang, C.-J., and Smith, D. L.,. . .

DETD Plant materials for Compounds 3-5. The **bark** of Asimino triloba (L.) Dunal was collected from stands growing wild at the Purdue Horticultural Research Farm. The identification was confirmed by Dr. George R. Parker, Department of Forestry and Natural Resources, Purdue University. A voucher specimen of the **bark** is preserved in the pharmacognosy herbarium.

DETD Extraction and purification. The air-**dried** **pulverized** stem **bark** (15 kg) was extracted exhaustively with 95% EtOH and vacuum evaporated to yield extract FO01 (1645 g) which was partitioned. . .

DETD **Goniothalamus** giganteus Hook. f. et Thomas (Annonaceae) is a tropical tree widely distributed in southeast Asia. Extracts of the **bark**, obtained from Thailand, showed toxicities in the brine shrimp test (BST) and showed murine toxicities in the 3PS (P388) leukemia bioassay. From the **ethanol** extract of the **bark**, eleven highly cytotoxic Annonaceous acetogenins have been isolated and, among them, four have a double bond in the aliphatic chain.. . . relatively rare feature in the Annonaceous acetogenins. Over 90 acetogenins have been described, yet, only one additional acetogenin, bullatenin from **Annona** bullata, has been found having a double bond in the chain. We have isolated from the **bark** of G. giganteus a new mono-THF acetogenin, gonionenin, which also has a double bond in the aliphatic chain. The C-21/22. . .

DETD . . . isolated from G. giganteus and contains a mono-THF and an isolated chain double bond. Recently, 28 was also found in **Xylopia** ##STR13##

DETD Plant Material. The stem **bark** of G. giganteus (B-826538, PR-50604) was collected in Thailand in September 1978 under the auspices of Dr. Robert E. Perdue,. . .

DETD **Extraction** and Isolation. The residue of the 95% EtOH **crude extract** of 4 kg of the stem **bark** was partitioned between H.sub.2 O and CHCl.sub.3 to give an H.sub.2 O layer and a CHCl.sub.3 layer. The residue of. . . was partitioned between hexane and 10 % H.sub.2 O in MeOH to give a MeOH layer (ca. 100 g of **dry** residue) and a hexane layer. The MeOH residue, which represented the most active fraction in the BST test (LC.sub.50 15.1. .

DETD . . . The mixture was washed using 1% NaHCO.sub.3 (5 ml) and H.sub.2 O (2+5 ml), and the CH.sub.2 Cl.sub.2 layer was **dried** in vacuo to give the 21/22-epoxide of 28; to the 21/22 epoxide (in 10 ml of CH.sub.2 Cl.sub.2) was added. . . The mixture was washed using 1% NaHCO.sub.3 (5 ml) and H.sub.2 O (2+5 ml), and the CH.sub.2 Cl.sub.2 layer was **dried** in vacuo and resolved by HPLC to give 35 mg of 29 (yield: 38%) and 35 mg of 22 (yield:. . .

DETD An additional acetogenin, Goniocin, was isolated from the **ethanolic** extracts of the **bark** of **Goniothalamus** giganteus after partitionings and repeated chromatographic separations. Lethality of the fraction to the larvae of brine shrimp in the brine shrimp lethality test was used to guide the fractionation. The residue of the 95% EtOH **crude** **extract** of 4 kg of the stem **bark** was partitioned between H.sub.2 O and CHCl.sub.3. The residue of the CHCl.sub.3 layer

was partitioned between hexane and 10% H.sub.2 O in MeOH to give 100 g of **dry** MeOH residue. The MeOH residue, which represented the most active fraction in the BST test (LC.sub.50 15.1 μg/ml), was repeatedly. . .

DETD Mono-**alcohols** can be converted into intermolecular formaldehyde acetals using chlorotrimethylsilane (Me.sub.3 SiCl ) and dimethyl sulfoxide (Me.sub.2 SO). Bal, B.S. and. . .

DETD . . . 44, p. 3727 (1979)) that monoalcohols can be converted to intermolecular formaldehyde acetals derivatives by mixing equivalent millimolar concentrations of mono-**alcohols**, Me.sub.3 SiCl, and Me.sub.2 SO. However, applicants found that adding equivalent millimolar concentrations of Me.sub.3 SiCl and Me.sub.2 SO to. . .

DETD . . . were available as isolated in our laboratory from several plant species in the Annonaceae. Squamostatin A (13) was isolated from **Annona** squamosa and provided by Bayer AG, Germany.

DETD . . . The mixture was washed using 1% NaHCO.sub.3 (5 ml) and H.sub.2 O (2+5 ml), and the CH.sub.2 Cl.sub.2 layer was **dried** in vacuo. The products were purified by normal phase open column chromatography (0.5% MeOH in CHCl.sub.3) or HPLC [5-10% MeOH:THF. . .

DETD . . . pipet (0.6+6 cm) containing silica gel (60-200 mesh) and eluted with 3 ml of CH.sub.2 Cl.sub.2. The CH.sub.2 Cl.sub.2 residue, **dried** in vacuo, was redissolved in CH.sub.2 Cl.sub.2 and washed using 1% NaHCO.sub.3 (5 ml) and H.sub.2 O (2+5 ml); the CH.sub.2 Cl.sub.2 layer was **dried** in vacuo to give the S-Mosher esters. Using S-(+)-α-methoxy-α-(trifloromethyl)phenylacetyl chloride gave the R-Mosher esters. Both yields were typically higher than. . .

DETD . . . 3 and State 4 respirations were allowed to stabilize. Next, 10 μl of the freshly prepared acetogenin solution (in 95% **ethanol**) was injected, and the solution was allowed to equilibrate for 2 minutes. After equilibration, 5 μl of ADP was added,. . .

| | |
|---|---|
| ACCESSION NUMBER: | 96:63234 USPATFULL |
| TITLE: | Bioactive acetogenins and derivatives |
| INVENTOR(S): | McLaughlin, Jerry L., West Lafayette, IN, United States<br>Gu, Zhe-ming, West Lafayette, IN, United States<br>Zhao, Geng-xian, West Lafayette, IN, United States |
| PATENT ASSIGNEE(S): | Purdue Research Foundation, West Lafayette, IN, United States (U.S. corporation) |

| | NUMBER | KIND | DATE | |
|---|---|---|---|---|
| PATENT INFORMATION: | US 5536848 | | 19960716 | |
| APPLICATION INFO.: | US 1994-259383 | | 19940614 | (8) |
| DOCUMENT TYPE: | Utility | | | |
| FILE SEGMENT: | Granted | | | |
| PRIMARY EXAMINER: | Trinh, Ba Kim | | | |
| LEGAL REPRESENTATIVE: | Barnes & Thornburg | | | |
| NUMBER OF CLAIMS: | 2 | | | |
| EXEMPLARY CLAIM: | 1,2 | | | |

L21 ANSWER 16 OF 18 USPATFULL on STN

AB Novel acetogenins isolated from **Asimina** triloba and **Goniothalamus** giganteus of the family Annonaceae and derivatives of those and other acetogenins are described. Bioactive cyclic formaldehyde acetal derivatives are. . . having 1,2-, 1,4- or 1,5-diols. A non-adjacent bis-tetrahydrofuran (THF) acetogenin is prepared from an unsaturated mono-THF acetogenin earlier isolated from **Goniothalamus** giganteus. The substantially pure acetogenins and acetogenin derivatives of the invention exhibit cytotoxicity to human solid tumor cell lines equipotent. . .

SUMM . . . in the phyto-chemistry of the Annonaceae has been sparked by the bioactivity-directed isolation of the antileukemic Annonaceous acetogenin, uvaricin, from **Uvaria** acuminata. Acetogenins are C.sub.35 -C.sub.39 compounds and typically contain two long hydrocarbon chains, one of which connects a terminal 2,4-disubstituted-γ-lactone. . .

SUMM . . . present invention there are provided novel, cytotoxic acetogenins and acetogenin derivatives. One group of acetogenins of this invention isolated from **Asimina** triloba are represented by the general formula

SUMM . . . thereof. The compound wherein --R.sub.3 -- is the divalent group (VI) is denominated goniocin, a naturally occurring acetogenin isolated from **Goniothalamus** giganteus. The compound wherein --R.sub.3 -- is the divalent group (VII) is prepared by epoxidation and subsequent acid-catalyzed cyclization of a previously reported acetogenin, gigantetronenin (also isolated from **Goniothalamus** giganteus), having the above formula wherein --R.sub.3 -- is a divalent group of the formula ##STR5##

DETD Bullatacin is one of the most potent antitumor and pesticidal Annonaceous acetogenins and was first reported and isolated from **Annona** bullata in 1989. The correct absolute configurations of the stereogenic carbinol centers of bullatacin were recently established by .sup.1 H-. . . systems to reduce the ATP levels. Eleven Annonaceous acetogenins have been previously reported from the ETOH extract of the stem **bark** of **Asimina** triloba.
Directed by the brine shrimp lethality test (BST). Two related isomeric acetogenins, the bullatacin threo-trans-threo-trans-erythro from C-15 to C-24. . .

DETD The **bark** of **Asimina** triloba (L.) Dunal was collected from stands growing wild at the Purdue Horticultural Research Farm, West Lafayette, Ind., U.S.A. The. . . was confirmed by Dr. George R. Parker, Department of Forestry and Natural Resources, Purdue University. A voucher specimen of the **bark** is preserved in the pharmacognosy herbarium.

DETD The air-**dried pulverized** stem **bark** (15 kg) was extracted exhaustively (12 days) at room temperature with 95% EtOH (451+4) and vacuum evaporated to yield extract. . .

DETD Asimicin was the first acetogenin isolated from the **seeds** and stem **bark** of the North American paw paw tree, **Asimina** triloba Dunal (Annonaceae). Asimicin has been reported as exhibiting highly potent antitumor and pesticidal activities. Further studies of A. triloba stem **bark** has led to the discovery of additional novel bioactive acetogenins, including the very active adjacent bis-tetrahydrofuran (THF) compound, trilobacin. The. . .

DETD Further activity-directed fractionation of the **ethanolic** extract of the stem **bark**, using the brine shrimp lethality test (BST) to monitor fractionation, has revealed three novel adjacent bis-THF acetogenins, asimin (3), asiminacin. . .

DETD In searching for new bioactive acetogenins from the F005 fraction, which was partitioned from the EtOH extract of the stem **bark**, the more polar column fractions from the most active pools (P7-P9) were investigated. The fraction sample was subjected to open. . .

DETD . . . EtOH and heating. Chromatotron plates (1 or 2 mm) were prepared with silica gel 60 PF 254 containing gypsum and **dried** at 700

overnight. HPLC was carried out with a Rainin HPLC instrument using the Dynamax software system and a silica. . .

DETD . . . the presence of pyridine. Approximately 10-50 μg of pure compound was placed in a 100 μl conical reaction vial and **dried** in a vacuum desiccator over P.sub.2 O.sub.5 for 24 hrs. The sample was treated with 2 μl pyridine and 20. . .

DETD . . . X.-P.; Miesbauer, L. R., Smith, D. L.; and McLaughlin, J. L., 30, 31, and 32-Hydroxybullatacinones: Bioactive Terminally-Hydroxylated Annonaceous Acetogenins from **Annona** bullata., J. Nat. Prod., 1993, 56, 870-876; MCF-7 (human breast carcinoma) McLaughlin, J. L., Chang, C.-J., and Smith, D. L.,. . .

DETD Plant materials for Compounds 3-5. The **bark** of Asimino triloba (L.) Dunal was collected from stands growing wild at the Purdue Horticultural Research Farm. The identification was confirmed by Dr. George R. Parker, Department of Forestry and Natural Resources, Purdue University. A voucher specimen of the **bark** is preserved in the pharmacognosy herbarium.

DETD Extraction and purification. The air-**dried** **pulverized** stem **bark** (15 kg) was extracted exhaustively with 95% EtOH and vacuum evaporated to yield extract F001 (1645 g) which was partitioned. . .

DETD **Goniothalamus** giganteus Hook. f. et Thomas (Annonaceae) is a tropical tree widely distributed in southeast Asia. Extracts of the **bark**, obtained from Thailand, showed toxicities in the brine shrimp test (BST) and showed murine toxicities in the 3PS (P388) leukemia bioassay. From the **ethanol** extract of the **bark**, eleven highly cytotoxic Annonaceous acetogenins have been isolated and, among them, four have a double bond in the aliphatic chain.. . . relatively rare feature in the Annonaceous acetogenins. Over 90 acetogenins have been described, yet, only one additional acetogenin, bullatenin from **Annona** bullata, has been found having a double bond in the chain. We have isolated from the **bark** of G. giganteus a new mono-THF acetogenin, gonionenin, which also has a double bond in the aliphatic chain. The C-21/22. . .

DETD . . . isolated from G. giganteus and contains a mono-THF and an isolated chain double bond. Recently, 28 was also found in **Xylopia** ##STR13##

DETD Plant Material. The stem **bark** of G. giganteus (B-826538, PR-50604) was collected in Thailand in September 1978 under the auspices of Dr. Robert E. Perdue,. . .

DETD **Extraction** and Isolation. The residue of the 95% EtOH **crude extract** of 4 kg of the stem **bark** was partitioned between H.sub.2 O and CHCl.sub.3 to give an H.sub.2 O layer and a CHCl.sub.3 layer. The residue of. . . layer was partitioned between hexane and 10% H.sub.2 O in MeOH to give a MeOH layer (ca. 100 g of **dry** residue) and a hexane layer. The MeOH residue, which represented the most active fraction in the BST test (LC.sub.50 15.1. . .

DETD . . . The mixture was washed using 1% NaHCO.sub.3 (5 ml) and H.sub.2 O (2+5 ml), and the CH.sub.2 Cl.sub.2 layer was **dried** in vacuo to give the 21/22-epoxide of 28; to the 21/22 epoxide (in 10 ml of CH.sub.2 Cl.sub.2) was added. . . The mixture was washed using 1% NaHCO.sub.3 (5 ml) and H.sub.2 O (2+5 ml), and the CH.sub.2 Cl.sub.2 layer was **dried** in vacuo and resolved by HPLC to give 35 mg of 29 (yield: 38%) and 35 mg of 22 (yield:. . .

DETD An additional acetogenin, Goniocin, was isolated from the **ethanolic** extracts of the **bark** of **Goniothalamus** giganteus after partitionings and repeated chromatographic separations. Lethality of the fraction to the larvae of brine shrimp in the brine shrimp lethality test was used to guide the fractionation. The residue of the 95% EtOH **crude** **extract** of 4 kg of the stem **bark** was partitioned between H.sub.2 O and CHCl.sub.3. The residue of the CHCl.sub.3 layer was partitioned between hexane and 10% H.sub.2 O in MeOH to give 100 g

of **dry** MeOH residue. The MeOH residue, which represented the most active fraction in the BST test (LC.sub.50 15.1 μg/ml), was repeatedly. . .

DETD Mono-**alcohols** can be converted into intramolecular formaldehyde acetals using chlorotrimethylsilane (Me.sub.3 SiCl) and dimethyl sulfoxide (Me.sub.2 SO). Bal, B. S. and. . .

DETD . . . 44, p. 3727(1979)) that monoalcohols can be converted to intramolecular acetogenins formaldehyde acetyl derivatives by mixing equivalent millimolar concentrations of mono-**alcohols**, Me.sub.3 SiCl, and Me.sub.2 SO and converted the mono-**alcohols** into intermolecular formaldehyde acetals. However, applicants found that adding equivalent millimolar concentrations of Me.sub.3 SiCl and Me.sub.2 SO to acetogenins. . .

DETD . . . were available as isolated in our laboratory from several plant species in the Annonaceae. Squamostatin A (13) was isolated from **Annona** squamosa and provided by Bayer AG, Germany.

DETD . . . The mixture was washed using 1% NaHCO.sub.3 (5 ml) and H.sub.2 O (2+5 ml), and the CH.sub.2 Cl.sub.2 layer was **dried** in vacuo. The products were purified by normal phase open column chromatography (0.5% MeOH in CHCl.sub.3) or HPLC [5-10% MeOH:THF. . .

DETD . . . pipet (0.6+6 cm) containing silica gel (60-200 mesh) and eluted with 3 ml of CH.sub.2 Cl.sub.2. The CH.sub.2 Cl.sub.2 residue, **dried** in vacuo, was redissolved in CH.sub.2 Cl.sub.2 and washed using 1% NaHCO.sub.3 (5 ml) and H.sub.2 O (2+5 ml); the CH.sub.2 Cl.sub.2 layer was **dried** in vacuo to give the S-Mosher esters. Using S-(+)-α-methoxy-α-(trifloromethyl)phenylacetyl chloride gave the R-Mosher esters. Both yields were typically higher than. . .

DETD . . . 3 and State 4 respirations were allowed to stabilize. Next, 10 μl of the freshly prepared acetogenin solution (in 95% **ethanol**) was injected, and the solution was allowed to equilibrate for 2 minutes. After equilibration, 5 μl of ADP was added,. . .

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|---|---|
| ACCESSION NUMBER: | 1998:14952 USPATFULL |
| TITLE: | Bioactive acetogenins and derivatives |
| INVENTOR(S): | McLaughlin, Jerry L., West Lafayette, IN, United States<br>Gu, Zhe-ming, West Lafayette, IN, United States<br>Zhao, Geng-xian, West Lafayette, IN, United States |
| PATENT ASSIGNEE(S): | Purdue Research Foundation, West Lafayette, IN, United States (U.S. corporation) |

| | NUMBER | KIND | DATE |
|---|---|---|---|
| PATENT INFORMATION: | US 5717113 | | 19980210 |
| APPLICATION INFO.: | US 1996-679005 | | 19960712 (8) |
| RELATED APPLN. INFO.: | Division of Ser. No. US 1994-259383, filed on 14 Jun 1994, now patented, Pat. No. US 5536848 | | |

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|---|---|
| DOCUMENT TYPE: | Utility |
| FILE SEGMENT: | Granted |
| PRIMARY EXAMINER: | Trinh, Ba K. |
| LEGAL REPRESENTATIVE: | Barnes & Thornburg |

ANSWER 15 OF 18 USPATFULL on STN

AB The isolation process to obtain physalins comprises the steps of: (a) grinding roots, leaves or stem-**bark** of Physalis ssp; (b) extracting the material obtained in step (a) with solvents selected from the group consisting of water and **alcohols**, such as methanol, **ethanol**, 1-propanol, 2-propanol, isobutanol; (c) evaporating the extract obtained in step (b) and washing the syrup material with a suitable solvent;. . .

SUMM [0005] **Crude extracts** from Physalis species are reported to have been used in indigenous medicine systems. It is also mentioned by Sanchez et. . . extracts and their isolated constituents exhibit biological activity, including the anti-bacterial effect of P. angulata extracts from root, leaf and stem-**bark**.

SUMM . . . (sleeping sickness) caused by Trypanosoma brucei rhodesiense. The most active extracts with IC.sub.50 values below 1 μg/ml were derived from **Annona** senegalensis, Bussea occidentalis and Physalis angulata. Compared to IC.sub.50 values of commonly used trypanocidal drugs, e.g. suramin at a concentration of 10.7 ng/ml, the values even for active **extracts** were high. However, since the **crude** plant **extracts** used are mixtures of various compounds, purification of active extracts might result in a considerable increase in activity.

SUMM [0023] U.S. Pat. No. 5,290,553 describes alkaloid extracts from **seeds**, **fruit**-rind and stem-**bark** and new isolated alkaloids from Picralima nitida, and alkaloid extracts from **seeds**, **fruit**-rind and stem-**bark** of plants selected from the group consisting of Gongronema latifolia, Rothmania withfieldii and Desmodium gangeticum used for the treatment of. . .

SUMM [0028] The object of the present invention is the use of ergostane-type steroids, named physalins, and to **alcoholic** and aqueous extracts from Physalis species in the treatment of infections caused by protozoans. As immunomodulators, physalins and Physalis extracts. . .

SUMM [0031] Other embodiment of the present invention provides a pharmaceutical composition having an **alcoholic** steroid extract from Physalis species combined with a pharmaceutically acceptable carrier.

SUMM . . . embodiment, the present invention provides an isolation process to obtain physalins comprising the steps of: (a) grinding roots, leaves or stem-**bark** of Physalis ssp; (b) extracting the material obtained in step (a) with solvents selected from the group consisting of water and **alcohols**, such as methanol, **ethanol**, 1-propanol, 2-propanol, isobutanol; (c) evaporating the extract obtained in step (b) and washing the residue with a suitable solvent; (d). . .

DETD . . . being collected, roots or epigeal parts of P. angulata may be cut in small pieces and ground in a mixer. **Crude extract** is treated with an aqueous or **alcoholic** solvent in a suitable extractor, at room or higher temperature, the later by heating for at least 24 hours. Suitable **alcoholic** solvents include, but are not limited to methanol, **ethanol**, 1-propanol, 2-propanol, iso-butanol, sec-butanol and the like. The **alcoholic** extract when tested for anti T. cruzi activity showed 100% of mortality. The aqueous or organic extract is further evaporated. . .

DETD [0057] 330 g of **dried** roots of P. angulata were cut in small pieces, ground and extracted with **ethanol** by heating in a Soxhlet extractor. The obtained extract was concentrated to **dryness** under reduced pressure and the resulting syrup material was washed with chloroform, in a proportion of about 3 to 5. . .

DETD . . . these animals were daily, since the day before infection, treated with 20 mg/animal of (a) methanolic extract obtained from the **fruit** of P. angulata L.; (b) physalin mixture (physalins B, D, G, H and L) obtained from the leaves of P. angulata L.; and (c) **ethanolic** extract obtained from the stem-**bark** of P. angulata L. The treatment was orally applied.

DETD . . . B, D, G, H 33
and L) obtained from the leaves of P. angulata
Methanolic extract obtained from the 35
**fruit** of P. angulata
**Ethanolic** extract obtained from the 16
stem-**bark** of P. angulata L

DETD [0078] From Table II, it can be concluded that the best efficacy performance (84%) was obtained when **ethanolic** extract from the stem-**bark** of P. angulata L. is applied in the treatment. The second best result (efficacy of 67%) is in the treatment. . . B, D, G, H and L) from the leaves of P. angulata L. The treatment with methanolic extract from the **fruit** of P. angulata L. showed an efficacy of 65%.

CLM What is claimed is:
9) A pharmaceutical composition comprising an effective amount of an **alcoholic** steroid extract from Physalis species in combination with a pharmaceutically acceptable carrier.

11) The pharmaceutical composition according to claim 9 wherein the **alcoholic** steroid extract is derived from P. angulata.

. . . isolation process of physalin from plants belonging to the Solonaceae family comprising the steps of: (a) grinding roots, leaves or stem-**bark** of Physalis ssp; (b) extracting the material obtained in step (a) with solvents selected from the group consisting of water and **alcohols**, such as methanol, **ethanol**, 1-propanol, 2-propanol, isobutanol; (c) evaporating the extract obtained in step (b) and washing the syrup material with a suitable solvent;. . .
17) The isolation process according to claim 15 wherein the heating extraction is conducted in a suitable extractor with **ethanol** for at least 24 hours.


ACCESSION NUMBER: 2002:192312 USPATFULL
TITLE: PROCESS FOR ISOLATING PHYSALINS FROM PLANTS AND PHARMACEUTICAL COMPOSITIONS CONTAINING PHYSALINS


INVENTOR(S): TOMASSINI, THEREZINHA C. B., RIO DE JANEIRO, BRAZIL
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SOARES, MILENA B. P., SALVADOR BAHIA, BRAZIL
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| | NUMBER | KIND | DATE |
|---|---|---|---|
| PATENT INFORMATION: | US 2002103386 | A1 | 20020801 |
| APPLICATION INFO.: | US 1999-417779 | A1 | 19991014 (9) |

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Nixon & Vanderhye PC, 1100 North Glebe Rd, 8th Floor, Arlington, VA, 22201-4714
NUMBER OF CLAIMS: 28
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 9 Drawing Page(s)
LINE COUNT: 703
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

isolated two
compounds designated as squamocin and neoannonin possessing insecticidal activity from the **seeds** of **Annona** squamosa in the following steps; (1), air **dried** ground **seeds** of **Annona** squamosa_(2.1 kg) was subjected to extractions with (a), hexane and (b) ethyl acetate successively. The concentrate of the active ethyl. . .

DETD [0101] The new compound isosquamocin, the standardized extract of the **seeds** of **Annona** squamosa (SESAS) containing isosquamocin (1) and related compounds squamocin-G (2), asimicin/squamocin-H, (3), 4-deoxyasimicin/squamocin-M (4), desacetyluvarian/squamocin-L (5), motrilin/annonin-III/squamocin-C (6), neoannonin /squamocin-J. . . retention times 5.88 min, 14.18 min ,and 45.25 min. in HPLC and the formulations of the standardized extract of the **seeds** of **Annona** squamosa have been found to possess biological properties which enable them to be used for the control of insect pests. . . .

DETD . . . found that isosquamocin and the related compounds (2) to (12) and three unidentified compounds and the standardized extract of the **seeds** of **Annona** squamosa (SESAS) consisting of isosquamocin (1) and related compounds (2) to (12) and three unidentified compounds is prepared by a process in which the **seeds** of **Annona** squamosa are used in the following steps.

DETD [0103] 1. **Dry seeds** are disintegrated into a coarse powder in a multimill (Gannon private Ltd, Bombay, India);

DETD [0104] 2. The **seed** powder from step 1 is packed into a column and **percolated** continuously with water miscible aliphatic **alcohols** such as methanol, **ethanol**, n-propanol and suitable compositions of these solvents with water and the extract is concentrated;

DETD . . . The organic solvent phases are combined and concentrated resulting in oily residue which is designated as standardized extract of the **seeds** of **Annona** squamosa (SESAS) and its HPLC is shown in FIG. 5. SESAS has been found to contain isosquamocin upto 48% and. . .

DETD [0108] 6. Alternatively the **seed** powder from step 1 is packed into a column and continuously **percolated** at ambient temperature with solvents such as benzene, dichloromethane, chloroform dichloroethane, ethylacetate, acetone, 2-butanone, methyl tertiary butyl ether, disopropyl ether,. . . petroleum ether b.p.60-80° C./hexane/pentane and the supernatant liquid is decanted and discarded. The resulting semisolid is free of solvents by **drying** at atmospheric pressure or reduced pressure, and it is the standardized extract of the **seeds** of **Annona** squamosa (SESAS) containing upto 57% of isosquamocin and compounds (2) to (15) and its HPLC is shown in FIG. 5.

DETD [0109] 7. The standardized extract of **seeds** of **Annona** squamosa from step (5) or (6) is subjected to column chromatography (Sigel) using petroleumether, b.p. 60-80° C., petroleumether/ethyl acetate, 9/1. . .

DETD [0111] 9. The standardized extract of the **seeds** of **Annona** squamosa prepared by steps (5) or (6) is stirred with solvents (such as toluene xylene C-IX, aromax, **ethanol**, n-propanol, isopropanol, n-butanol, isobutanol, 2-butanone methylisobutylketone, cyclohexanone, ethylacetate, dimethyl phthalate, di n-octyphthalate, acetonitriledimethyl sulfoxide, dioxan, dimethyl formamide, water or suitable. . .

DETD . . . compounds, such as insecticides, acarcides, nematicides, fungicides growth promoters and herbicides. These insecticides include for examples azadirachtin, azadirachtin containing neem **seed** extract and other pesticidel plant extracts. Bacillus thuringiensis, other synthetic pesticides like organic carbamates, organophsphates, phenyl ureas, pyrethroids and substances. . .

DETD [0114] The preparation of standardized extract of the **seeds** of **Annona** squamosa (SESAS), its active principles and their formulations are illustrated with the aid of the following examples and they do. . .

DETD [0115] The **seeds** of **Annona** squamosa (10 kg) were disintegrated in a multimill (Gannon Private Ltd., Bombay, India) to a coarse powder having particle size. . . mm) to BSS-72 (2.4 mm) and the powder was packed into a glass column. The column containing the powder of **seeds** of **Annona** squamosa was then continuously extracted by **percolation** with methanol (40 l) at ambient temperature. The resulting extract (36 l) was concentrated at atmospheric pressure or under reduced. . . removed at atmospheric pressure or under reduced pressure resulting in a brown semisolid which was designed as standardized extract of **seeds** of **Annona** squamosa (SESAS) (200 g) and it was found contain 38.89% of isosquamocin by analytical HPLC using a reverse phase C.sub.18. . .

DETD [0118] The residual **seed** powder (9,35 kg) after extraction of methanol was stripped of the adhering solvents packed again into the glass column and **percolated** with petroleum ether (b.p.6-80° C.)/hexane (401.) continuously and the petroleum ether b.p. 60-80° C./hexane extract was concentrated at atmospheric pressure or under reduced pressure resulting in an oil (1.8 kg). The remaining powder of **seeds** of **Annona** squamosa after the extraction with hexane was stripped the solvent and it weighed 7.3 kg.

DETD [0119] The **seeds** of **Annona** squamosa (200 g) were disintegrated in a multimill (Gannon Private Ltd. Bombay, India) to a coarse powder having participle size. . . to BSS-72 (2.4 mm) and the powder was packed into a glass column. The column packed with the powder of **seeds** of **Annona** squamosa was then continuously extracted by **percolation** with methanol (1000 ml) at ambient temperature. The resulting extract (890 ml) was concentrated at atmospheric pressure or under reduced. . . in a brown viscous mass containing 38.89% of isoannonin (2.789 g) and this also constitutes the standardized extract of the **seeds** of **Annona** squamosa (SESAS). Its analytical HPLC is similar to that of the corresponding sample obtained in example-1. This sample was subjected. . .

DETD [0120] The **seeds** of **Annona** squamosa (10 kg) were disintegrated in a multimill (Gannon Private Ltd., Bombay, India) to a coarse powder having particle size. . . mm) to BSS-72 (2.4 mm) and the powder was packed into a glass column. The column containing the powder of **seeds** of **Annona** squamosa was then continuously extracted by **percolation** with **ethanol** (40 lit) at ambient temperature. The resulting extract (36 lit) was concentrated at atmospheric pressure or under reduced pressure and. . . removed at atmospheric pressure or under reduced pressure resulting in a brown semisolid which was designed as standardized extract of **seeds** of **Annona** squamosa (SESAS) (200 g) and it was found to contain 38.89% of isosquamocin by analytical HPLC using a reverse phase. . .

DETD [0123] The residual **seed** powder (9.35 kg) after extraction of **ethanol** was stripped of the adhering solvents packed again into the glass column and **percolated** with petroleum ether (b.p.6-80° C.)/hexane (40 lit) continuously and the petroleum ether b.p. 60-80° C./hexane extract was concentrated at atmospheric pressure or under reduced pressure resulting in oil (1.8 kg). The remaining powder of **seeds** of **Annona** squamosa after the extraction with hexane was stripped the solvent and it weighed 7.3 kg.

DETD [0124] The **seeds** of **Annona** squamosa (200 g) were disintegrated in a multimill (Gannon Private Ltd. Bombay, India) to a coarse powder having participle size. . . to BSS-72 (2.4 mm) and the powder was packed into a glass column. The column packed with the powder of **seeds** of **Annona** squamosa was then continuously

extracted by **percolation** with **ethanol** (1000 ml) at ambient temperature. The resulting extract (890 ml) was concentrated at atmospheric pressure or under reduced pressure and. . . in a brown viscous mass containing 39.00% of isoannonin (2.72 g) and this also constitutes the standardized extract of the **seeds** of **Annona** squamosa (SESAS). Its analytical HPLC is similar to that of the corresponding sample obtained in example-1. This sample was subjected. . .

DETD [0125] The **seeds** of **Annona** squamosa (200 g) were disintegrated in a multimill (Gannon Private Ltd. Bombay, India) to a coarse powder having participle size. . . to BSS-72 (2.4 mm) and the powder was packed into a glass column. The column packed with the powder of **seeds** of **Annona** squamosa was then continuously extracted by **percolation** with methanol water (80/20) at ambient temperature. The resulting extract (890 ml) was concentrated at atmospheric pressure or under reduced. . . in a brown viscous mass containing 38.76% of isoannonin (2.68 g) and this also constitutes the standardized extract of the **seeds** of **Annona** squamosa. Its analytical HPLC is similar to that of the corresponding sample obtained in example-1. This sample was subjected to. . .

DETD [0126] The **seeds** of **Annona** squamosa (200 g) were disintegrated in a multimill (Gannon Private Ltd., Bombay, India) to a coarse powder having a particle. . . to BSS-72 (2.4 mm) and the powder was packed in a glass column. The column containing the powder of the **seeds** of **Annona** squamosa was continuously extracted with methanol/water (80/20) by **percolation** at ambient temperature. The resulting extract (890-ml) was concentrated at atmospheric pressure or under reduced pressure and the concentrate (20. . . was removed at atmospheric pressure or under reduced pressure resulting in a brown semisolid which was designated standardized extract of **seeds** of **Annona** squamosa (2.98 g) and it was found to contain 25% of isosquqamocin by analytical HPLC using a reverse phases C.sub.18. . .

DETD [0127] The **seeds** of **Annona** squamosa (200 g) were disintegrated in a multimill (Gannon Private Ltd., Bombay, India) to a coarse powder having a particle. . . to BSS-72 (2.4 mm) and the powder was packed in a glass column. The column containing the powder of the **seeds** of **Annona** squamosa was continuously extracted with **ethanol**/water (80/20) by **percolation** at ambient temperature. The resulting extract (890-ml) was concentrated at atmospheric pressure or under reduced pressure and the concentrate (20-ml). . . was removed at atmospheric pressure or under reduced pressure resulting in a brown semisolid which was designated standardized extract of **seeds** of **Annona** squamosa (3.0 g) and it was found to contain 29% of isosquamocin by analytical HPLC using a reverse phases C.sub.18. . .

DETD [0128] The **seeds** of **Annona** squamosa (200 g) were disintegrated in a multimill (Gannon Private Ltd., Bombay, India) to a coarse powder having a particle. . . to BSS-72 (2.4 mm) and the powder was packed in a glass column. The column containing the powder of the **seeds** of **Annona** squamosa was continuously extracted with **ethanol**/water (8/2) by **percolation** at ambient temperature. The resulting extract (890 ml) was concentrated at atmospheric pressure or under reduced pressure and the concentrate. . . was removed at atmospheric pressure or under reduced pressure resulting in a brown semi-solid which was designated standardized extract of **seeds** of **Annona** squamosa (2.95 g) and it was found to contain 30% of isosquamocin by analytical HPLC using a reverse phases C.sub.18. . .

DETD [0129] The **seeds** of **Annona** squamosa were disintegrated in a multimill (Gannon Private Ltd., Bombay, India) to a coarse powder having particle size ranging from. . . BSS-72 (2.4 mm) and the powder (100 g) was packed into a glass column. The column containing the powder of **seeds** of Annon squamosa was

continuously extracted by **percolation** in separate columns with the following solvents (500 ml) viz., ethyl acetate chloroform, dichlormethane, 1,2-dichloroethane, diethyl ether, diisopropyl ether, methyl. . . at atmospheric pressure or under reduced pressure resulting in a pale yellow oil, which constitutes the standardized extract of the **seeds** of **Annona** squamosa (SESAS). The isosquamocin content and the yield of the product are given in parenthesis for each of these solvents is as follows by this procedure **ethanol** (0.84 g, 36.87%), ethyl acetate (0.95 g, 43.80%), chloroform (0.92, 28.59%), dichloromethane (1.49, 32.76%), 1,2-dichloroethane (0.64 g, 32,46%), diethyl ether. . . 53.81%). The isosquamocin content was estimated by HPLC as described in example-1. The HPLC of the standardized extract of the **seeds** of **Annona** squamosa obtained by using all the solvents mentioned above were similar and contained squamocin-G, asimicin, 4-deoxyasimicin, desacetyluvaricin, motrilin, neoannonin, squamocin-B,. . .

DETD [0130] The **seeds** of **Annona** squamosa were disintegrated in a multimill to a coarse powder having a mesh size in the range BSS-7 (0.12 mm). . .

DETD [0134] The standardized extract of **seeds** of **Annona** squamosa containing 30% isosquamocin (SESAS) was stirred with cyclohexannone (70 g) and emulsifier (creslox 3409, 10 g) in a stirred. . .

DETD [0135] Standardized extract of **seeds** of **Annona** squamosa containing 30% isosquamocin (2 kg) was stirred with cyclohexanone (6.75 kg) the emulsifier (creslox 3409, 1 kg) and piperonyl. . .

DETD [0137] Standardized extract of **seeds** of **Annona** squamosa (20 g) containing 30% isosquamocin, solvent C-IX (65 g) and emulsifier (creslox 3433, 10 g) and piperonyl butoxide (5. . .

DETD [0138] Chromic larveal growth bioassay % of control given in parenthesis. Larval growth and larval survival of standardized extract of **seeds** of **Annona** squamosa (SESAS) (25%, 85%) and some of its components squamocin-G (34%, 45%) isosquamocin (20%, 55%), bullatalicin (135%, 100%) and squamostatin-A. . . The EC 50 and LC 50 squamocin-G were found to be 5.65 ppm and 12 ppm respectively. Standardized extract of **seeds** of **Annona** squamosa (SESAS) was also found to cause 50% mortality of black wire weevils (otiorhydnchus sulcatus of 0.5% (1 ul dose).. . .

DETD . . . to the lower surface of leaf discs (5cm.sup.2) cut from sorghum hybrid variety CSH5 and the discs were allowed to **dry** on filter papers. After **drying** the leaf, discs were offered to 10 first instar M. separata larvae in plastic cups or to one third instar. . .

DETD . . . No. Balanagar Luro Island

Bull. 42, 1163 Chem. 1994 53, 2719, 1989

Fujimoto et al.

Planta Medica 56, 312, 1990

4,689,232

Ground **seeds** Pullvarized extraction with **seeds** extracted hexane-extract with discarded Methanol (Mesh). . .

Ground **seeds** Ground **seeds** soxlet extracted extraction a) hexane and with Pet. b) ethylacetate Ether-allowed

Ground Ground **seeds** **seeds** Pet. extracted with Ether extraction

with ligroin

CLM What is claimed is:

. . . and unidentified compounds with retention times 5.88 min, 14.18 min and 45.25 min in HPLC, all derived from the plant **Annona** squamosa along with additives or carriers.

6. A process for the preparation of an extract of **seeds** of Anona squamosa standardized with respect to a novel active insecticidal compound designated as standardized extract of Isoquamocin and related compounds from **seeds** of Anona squamosa known as custard apple, said process comprises of: (a) disintegrating the custard apple **seeds** into powder, (b) subjecting the said powder of step (a) to continuous extraction with methanol or aqueous methanol, **ethanol** or aqueous **ethanol** at an ambient temperature, (c) concentrating the extract of step (b) and stirring the concentrate with petroleum ether/hexane having boiling. . .

7. The process of claim 6, wherein in step (a), the disintegration of the **seeds** is carried out in a mill.

8. The process of claim 6, wherein in step (b), the particle size of the disintegrated **seed** powder obtained is in the range of British Standard **Sieves** BSS-7 (0.2 mm) to BSS-72 (2.4 mm).

9. The process of claim 6, wherein in step (b), the solvents used for extracting disintegrated **seeds** is selected from methanol, aqueous methanol, **ethanol**, aqueous **ethanol** and most preferably methanol.

16. The process of claim 6, wherein in step (a), the powdered **seeds** are continuously **percolated** using a solvent at an ambient temperature through a glass column in which the powdered **seeds** are packed, concentrating the extract and stirring the extract so obtained with petroleum ether by 60-80° C. hexane/pentane; decanting and discarding the supernatant liquid to obtain a semisolid; and finally **drying** the semisolid at atmospheric pressure or reduced pressure to obtain standardized extract of the **seeds** of Anona squamosa (SESAS) having upto 57% of acetogenin comprising Isoquamocin, squamocin G, squamocin H, squamocin M, squamocin L, squamocin. . .

. . . 18. The process of claim 2, wherein said concentrate containing upto 6% isosquamocin is obtained by stirring standard extract of **seeds** of Anona squamosa (SESAS) having upto 57% autogenins consisting as a mixture of isosquamocin and related products with solvents or. . .

22. A stable emulsifiable concentrate containing upto 30% isosquamocin in a standardized extract of **seeds** of Anona squamosa (SESAS), is also used as insecticide or in any insecticidal formulation.

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|---|---|
| ACCESSION NUMBER: | 2003:72049 USPATFULL |
| TITLE: | Novel compound iso-squamocin obtained from **seeds** of **annona** squamosa and composition containing the same |
| INVENTOR(S): | Shankaram, Akella Venkata Bhavani, Hyderabad, INDIA<br>Murthy, Madugula Marthanda, Hyderabad, INDIA<br>Akkewar, Dattatreya Manohar, Hyderabad, INDIA<br>Subramanyam, Mukkamala, Hyderabad, INDIA<br>Rao, Attaluri Narasimha, Hyderabad, INDIA |

| | NUMBER | KIND | DATE |
|---|---|---|---|
| PATENT INFORMATION: | US 2003050336 | A1 | 20030313 |
| | US 6991818 | B2 | 20060131 |
| APPLICATION INFO.: | US 2002-107064 | A1 | 20020328 (10) |

| | NUMBER | DATE |
|---|---|---|
| PRIORITY INFORMATION: | US 2001-279681P | 20010330 (60) |

| | |
|---|---|
| DOCUMENT TYPE: | Utility |
| FILE SEGMENT: | APPLICATION |